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Allium cepa root meristem cells under osmotic (sorbitol) and salt (NaCl) stress *in vitro*

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Abstract – The effects of various concentrations of sorbitol (100, 200 and 360 mM) and NaCl (100, 200 and 300 mM) on root meristem cells of *in vitro*-cultured *Allium cepa* L. were analyzed after 10 and 20 days. Both root meristem cell cross-section area and nuclear volume decreased under osmotic and salt stress. The osmotic component of applied stresses had a greater impact on cell shrinkage, while ionic stress perturbed cell functioning, resulting in cell cycle arrest and various aberrations, affecting nucleus integrity. A concentration of 300 mM of NaCl in the culture medium caused complete inhibition of mitotic activity in onion root tip cells after 20 days of exposure. Analysis of the action of iso-osmotic concentrations of NaCl (200 mM) and sorbitol (360 mM) showed stronger mitodepressive effects of salt stress in comparison to osmotic stress.

Keywords: aberrations, cell size, mitotic index, nuclear volume, phase index, stress

Introduction

To study the effects of abiotic stress on plants, *in vitro* cultures are suitable, providing aseptic conditions, rigorous control of the physical environment and nutrition in the relatively small space needed for culture (Verslues et al. 2006, Kiełkowska et al. 2012). The stress in tissue culture can be induced by the addition of specific compounds to the growth medium. Based on their physiochemical properties, stress inducers can be divided into categories of ionic and cell penetrating (NaCl, KCl); non-ionic and penetrating (mannitol, sorbitol); and non-ionic and non-penetrating (polyethylene glycol (PEG)) (Gangopadhyay et al. 1997). Usually, non-ionic osmotic and water stresses are simulated by adding PEG, mannitol or sorbitol. Non-ionic osmotic stress refers to changes in any activity or integrity of plant cells due to an increase or decrease in the concentration of non-ionic solutes changing the water potential of the cell-surrounding solution (Ahmad et al. 2007). Ionic stress might be induced by NaCl or KCl, producing specific ionic toxicities; however, both salts also induce osmotic stress, due to the rise in salt concentration outside the cells, leading to inhibition of water uptake (Claeys et al. 2014). Osmotic stress under salinity affects plants immediately after the increase in salt concentration, while ionic stress develops later, together with the excessive accumulation of ions (Munns and Tester 2008, Deinlein et al. 2014).

Research on osmotically mediated water deficit has been focused mainly on the evaluation of its effects on germination, plant morphology, biomass production and biochemical processes (Bhargava and Paranjpe 2004, Grzesiak et al. 2006, Ahmad et al. 2007), and little attention has been devoted to its action at the cytological level. The effect of NaCl-induced stress has been investigated mostly in cereals, and results show that concentrations greater than 300 mM cause serious damage at the cellular level and lead to cell death (Munns and Tester 2008, Yumurtaci et al. 2009, Tabur and Demir 2010). Lower concentrations of NaCl reduced root growth, which was also reflected in the reduction of the size of the root meristem (Hanif and Davies 1998, Zadeh 2007). Recently, a reduction in root meristem size was also reported for wheat under PEG-mediated osmotic stress (Ji et al. 2014). However, the mechanism by which the stress affects the meristem length is not fully understood. It is related to the inhibition of cell division under stress conditions. West et al. (2004) suggested that stress rapidly blocks cell cycle progression to prevent entry into stages where the cell is vulnerable to damage, allowing the cellular defense system to be activated. Stress-induced inhibition of cell division leads to fewer cells being produced, which results in reduction in meristem size.

Kimball et al. (1975) reported that mannitol and sorbitol caused a reduction in the size of soybean cells in suspension cultures. Similar results were reported for epidermal

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cells of maize after NaCl treatment (Zörb et al. 2015). However, it is unclear whether the root meristem cell size is also affected under osmotic/salt stress conditions and whether the affected cells maintain the ability to divide. To evaluate these effects, it is necessary to perform observations for an extended period of time. Such an approach will ensure enough time for several rounds of cell cycle progression in stress conditions. Most available reports show the effects of salt/osmotic shock rather than stress, as results were collected within minutes to hours after application of stress conditions (Shavrukov 2013, Deinlein et al. 2014).

Onion is an important crop worldwide and is considered salt sensitive (Shannon and Grieve 1999). There are single studies covering the reaction of onion to salt stress at the cellular level (Bennici and Tani 2009, Chatterjee and Majumder 2010). Such information is needed to gain better knowledge concerning stress responses and stress tolerance mechanisms in glycophytes. This information has both fundamental and economic importance, as all vegetables are included in this group.

The goal of this study was to evaluate the cytogenetic effects of NaCl-mediated salt stress and sorbitol-mediated osmotic stress on onion root meristem cells. To reveal the effects of applied stresses on mitosis and cell and nuclear size, experiments were conducted for extended periods of 10 and 20 days. Analysis of the effects of iso-osmotic solutions of NaCl (200 mM) and sorbitol (360 mM) allows comparison of the impact of the osmotic and ionic compounds of the applied stresses on the tested features.

Materials and methods

Seeds of *Allium cepa* cv. Majka F1, (PlantiCo, Kraków, Poland) were surface-disinfected in 70% (v/v) ethanol for 5 min and in a 20% chloramine T (Biochemie Poland, Katowice, Poland) solution (w/v) for 20 min and then washed three times in sterile water, each for 5 min. For germination, seeds were placed in 500-mL plastic culture boxes (Pakler Lerka, Kraków, Poland) containing 80 mL of basal MS medium (Murashige and Skoog 1962) without growth regulators. Basal MS medium was prepared using a powdered MS salt mixture including vitamins (Duchefa, Uni-Market, Poznań, Poland) with 30 g L^{-1} sucrose and 0.8% (w/v) agar (Biocorp, Warszawa, Poland), pH 5.7−5.8. Cultures were maintained at 25±2 °C in darkness for germination. Boxes with seedlings were kept at 25±2 °C (16-h days, 55 µmol m^{-2} s^{-1}). Ten-day-old plants were placed in boxes with 80 mL of culture medium consisting of basal MS medium with addition of 100, 200 or 300 mM of NaCl and 100, 200 or 360 mM sorbitol. Solution of 360 mM sorbitol is iso-osmotic to solution of 200 mM NaCl. Plants grown in basal MS medium were used as controls. Plants were exposed to the media with given concentrations of NaCl and sorbitol for 10 or 20 days. At the end of exposure period, plants were removed from the culture boxes and prepared for observations.

Roots from *in vitro*-cultured plants were fixed in 96% ethanol/glacial acetic acid (3:1 v/v) and hydrolyzed with 1 N HCl for 10 min at 60 °C. Roots were then stained with Schiff's reagent (Sigma Aldrich Ltd., Poznań, Poland) for 1 h at room temperature. From roots, approximately1-mm-long root tips were excised and then squashed in 50 µL of acetocarmine. Observations of slides were performed under a Nikon Eclipse (Nikon Instruments Inc., Japan) light microscope. Microscopic analyses included measurements of interphasic cell area, interphasic nuclear volume (Vn), determination of the mitotic (MI) and phase (PI) indices, chromosomal aberration score, and nuclear abnormalities.

For the calculations of Vn and cell area, image analysis of interphase cells using Nikon imaging software NIS Elements Documentation was performed. Cell cross-section area was measured for 2000 interphase cells for each treatment. Results were expressed as the mean cell cross-section area in square micrometers. For Vn calculations, the radius (r, µm) of 2000 nuclei for each treatment was measured. The Vn was then calculated according to methods of Mesi and Kopliku (2013).

To study MI and aberrations, approximately 2000 cells per each of five slides per sample were analyzed. The MI was calculated as the number of cells in mitosis divided by the total number of cells × 100%. PI was calculated as the percentage of cells in a certain mitotic phase of division (prophase, metaphase, anaphase and telophase). In addition, the values of the metaphase/ana-telophase (M/A + T) ratios were calculated.

Data were analyzed using a factorial analysis of variance (ANOVA), and mean separations were conducted using a post-hoc Student−Newman−Keuls (SNK) test at $p \leq 0.05$. All results were expressed as the mean±standard error (SE) of the mean.

Results

Interphasic cell cross-section area

The average cross-section area of *in vitro*-grown onion root meristem cells was 407−413 µm², and it did not change significantly during the experiment (Tab. 1). A decrease in the cell cross-section area with increasing concentrations of stress agents during the time of exposure was observed. After the 10-day treatment, root meristem cells of plants grown on medium supplemented with 100 mM of sorbitol were 16% smaller than control cells during the same period of time, while after 20 days of exposure cells were 28% smaller. A similar tendency was observed in the roots of plants grown on the media with higher concentrations of sorbitol.

At day 10 of the experiment, the average cross-section area of meristem cells in onion roots cultured on medium with 100 mM of NaCl was 375.9±10.8 µm², which represented 92% of the area of control cells. At the same time, the cross-section area of meristem cells in roots cultured on medium with 300 mM of NaCl was reduced to a size representing 78% of the area of control cells. After 20 days of exposure to the salt stress, the decrease in cell area continued in parallel with salt concentration, and the meristem cells in roots grown on medium with 300 mM of NaCl were approximately half of the size of control cells.

Tab. 1. Mean cross-section area of *Allium cepa* root meristem cells exposed to sorbitol and NaCl *in vitro*. Means followed by the same letter are not significantly different (p≤0.05, Student−Newman−Keuls test).

Treatment (mM)		Exposure (days) 10 Cell area (μm² ± SE)	% to ctrl	20 Cell area (μm² ± SE)	% to ctrl
Control (ctrl.)		407.7±15.0 a	100	413.7±15.9 a	100
Sorbitol	100	341.5±8.4 c	84	296.5±11.0 de	72
	200	295.3±10.2 de	72	283.3±9.1 ef	68
	360	241.8±6.1 gh	59	218.6±7.0 hi	53
NaCl	100	375.9±10.8 b	92	258.7±27.1 fg	63
	200	335.7±16.5 c	82	238.0±12.4 gh	58
	300	319.6±72.0 cd	78	201.1±13.1 i	49

After 10 days of exposure of onion roots to iso-osmotic concentrations of sorbitol (360 mM) and NaCl (200 mM), a greater decrease in cell area was observed in sorbitol-treated roots; however, after 20 days of exposure, the mean areas of sorbitol- and salt-treated interphasic cells were similar: 218.6±7.0 μm² and 201.1±13.1 μm², respectively.

Interphasic nuclear volume

The mean Vn of onion root meristem cells in the control treatment was approximately 1440 μm³ (Tab. 2) and was relatively constant during the experiment. Sorbitol at a concentration of 100 mM had a minor effect on interphasic Vn in root tip cells; however, higher concentrations caused a decrease in Vn. The nuclear volume of root meristem cells exposed to 200 mM of sorbitol for 20 days was approximately 20% smaller than that of control cells. Exposure of root meristems to increasing concentrations of NaCl resulted in a decrease of Vn of treated cells compared to that of controls. The average Vn of root tip cells after 10-day exposure to medium with 100 mM of NaCl was similar to that of controls; however, the Vn decreased gradually with increasing salt concentration. The nuclei of root meristem cells exposed to 300 mM of NaCl represented 46% of the volume of nuclei in control cells. After 20 days of exposure to 360 mM of sorbitol, the volume of average nuclei in onion root meristem cells was 1045.0±29.0 μm³ (73% of Vn of control cells), while in the roots cultured on medium with iso-osmotic concentration of NaCl the volume of the nuclei was significantly reduced to 660.1±18.7 μm³ (51% of Vn of control cells).

Tab. 2. Nuclear volume (Vn) of *Allium cepa* root meristem cells exposed to sorbitol and NaCl *in vitro*. Means followed by the same letter are not significantly different (p≤0.05, Student−Newman−Keuls test).

Treatment (mM)		Exposure (days) 10 Vn (μm³ ± SE)	% to ctrl	20 Vn (μm³ ± SE)	% to ctrl
Control (ctrl)		1449.4±51.0 a	100	1428.3±46.1 a	100
Sorbitol	100	1458.1±55.0 a	101	1327.4±54.9 ab	93
	200	1189.8±45.2 bc	82	1147.2±43.4 bc	80
	360	987.1±39.4 c	68	1045.0±29.0 c	73
NaCl	100	1400.1±60.0 a	97	1106.0±36.6 bc	77
	200	1126.3±28.5 bc	78	660.1±18.7 d	51
	300	673.4±37.4 d	46	721.8±16.2 d	46

Mitotic activity

Mitosis in the roots of control plants was normal and undisturbed (Tab. 3, Figs. 1a−e). Alterations in MI were observed in the roots of treated plants depending upon the type of stress agent, its concentration and length of exposure. In the roots of plants grown on media with the addition of sorbitol, a decrease in mitotic activity compared to the controls was observed. During the entire experiment, the MI of sorbitol-treated cells was approximately 50% lower than the MI observed for control cells. Although not statistically significant, a decrease in MI with increasing concentration of sorbitol in the culture media and time of exposure was observed. Addition of NaCl to the culture medium caused a more severe decrease in mitotic activity of onion root tip cells. On medium with 100 mM of NaCl, the average number of mitotic figures was 25−60% lower than that of the controls and decreased with increasing concentration of salt and time of exposure. Twenty-day exposure to 300 mM of NaCl completely arrested cell divisions. The MI in onion roots grown for 10 and 20 days on medium with 360 mM of sorbitol was 14.8±1.6% and 16.4±3.2%,

Tab. 3. Mitotic (MI) and phase (PI) indices in *Allium cepa* root meristem cells exposed to sorbitol and NaCl *in vitro*. Means followed by the same letter are not significantly different (p≤0.05, Student−Newman−Keuls test). P – prophase, M – metaphase, A + T – anaphase and telophase.

Treatment (mM)		Exposure (days)	MI (% ± SE)	MI % to ctrl	PI P	PI M	PI A+T	PI M/A+T
Control (ctrl)		10	31.0±2.9 a	100	57.7	21.9	20.4	1.1
		20	28.2±1.4 a	100	53.7	23.6	22.7	1.0
Sorbitol	100	10	16.9±3.7 bcd	55	41.0	30.2	28.8	1.1
		20	15.1±1.2 bcd	53	49.8	25.8	24.4	1.1
	200	10	16.4±2.3 bcd	53	43.1	24.0	32.8	0.7
		20	17.7±2.9 bcd	63	46.7	24.6	28.8	0.9
	360	10	14.8±1.6 bcd	48	52.2	19.0	28.8	0.7
		20	16.4±3.2 bcd	58	42.0	25.7	32.3	0.8
NaCl	100	10	23.3±2.3 b	75	44.9	19.7	35.3	0.6
		20	12.0±2.1 cde	42	62.0	10.8	27.3	0.4
	200	10	10.4±1.3 cde	34	52.6	14.2	33.2	0.4
		20	7.5±1.6 df	27	37.8	15.2	47.0	0.3
	300	10	4.3±1.0 ef	14	41.7	16.7	41.7	0.4
		20	0.0±0.0 f	0	0.0	0.0	0.0	0.0

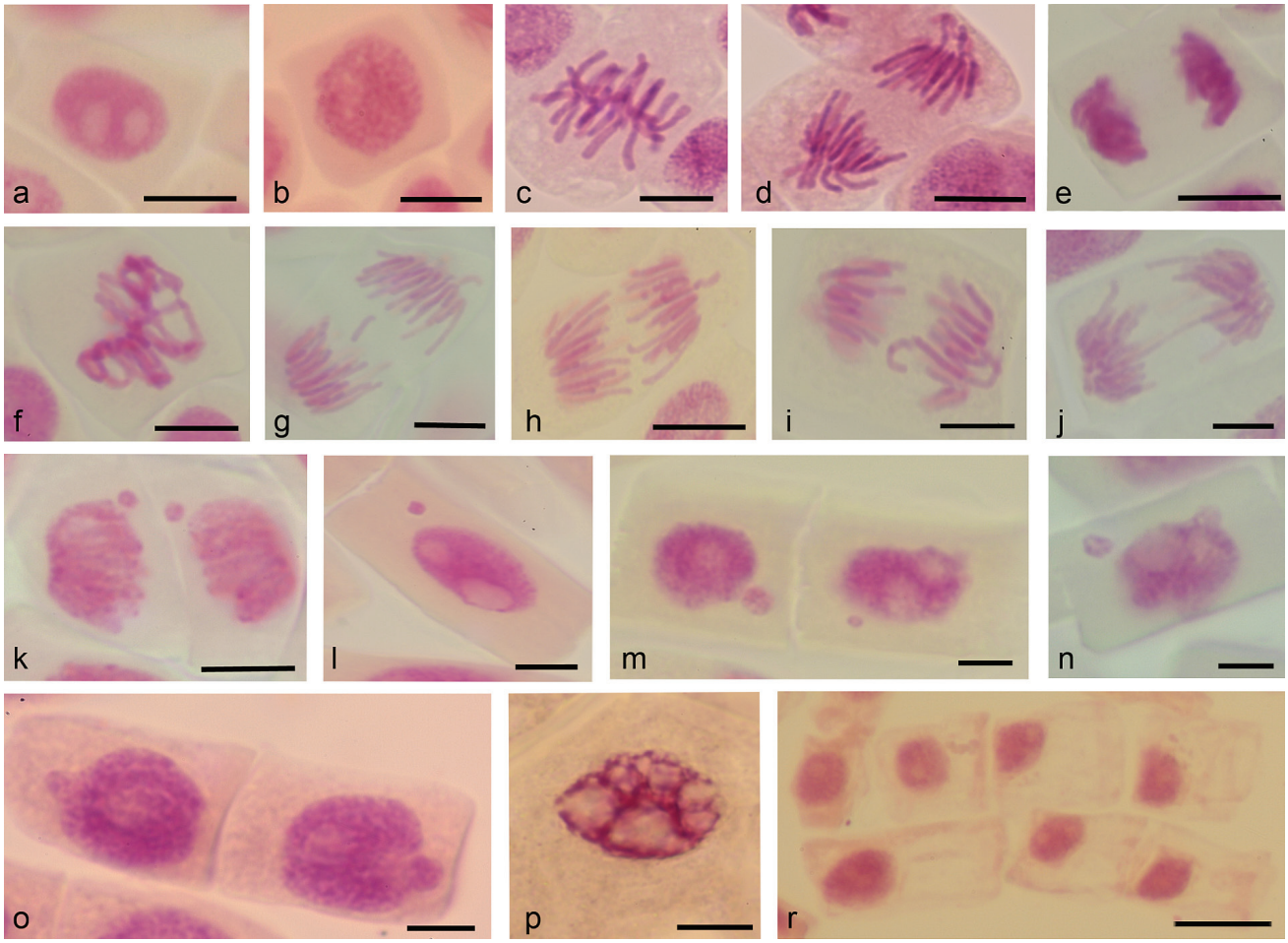

Fig. 1. *Allium cepa* root tip cells exposed *in vitro* to sorbitol and NaCl and control cells. Typical stages of mitosis in control roots: interphase (a), prophase (b), metaphase (c), anaphase (d) and telophase (e). Chromosomal aberrations after NaCl treatment: stickiness in metaphase (f) laggards, chromosome breaks and losses (g, h, i), chromosome bridge (j). Nuclear aberrations: micronuclei (k, l, m) and nuclear budding (n) in root tip cells treated with 200 mM NaCl; nuclear budding (o) in root tip cells treated with 360 mM sorbitol; nuclear disintegration (p) in root tip cells treated with 200 mM NaCl. Interphasic root tip cells after 20 days of treatment with 300 mM NaCl (r). Scale bar = 10 μm.

respectively, while in roots of plants grown on medium with iso-osmotic concentration of NaCl (200 mM) the MI was 10.4±1.3% and 7.5±1.6%, respectively. Microscopic observations showed that in the meristematic cells of roots exposed to 200 mM and 300 mM of NaCl, nuclei were shifted toward the cell wall (Fig. 1r). Moreover, in the roots of plants grown on medium with 300 mM of NaCl, in some of the observed meristem cells, despite staining, no nucleus was visible. In the control roots, the ratios of different mitotic phases after 10 and 20 days of growth were similar (Tab. 3). Most (54−58%) of the dividing root tip cells were in prophase, 22−24% were in metaphase and 20−23% were in ana/telophase, and the M/A + T ratio was approximately 1. Similar observations were made for roots treated with 100 mM of sorbitol. In the meristems of roots grown on media with higher concentrations of sorbitol, occurrences of anaphase and telophase were more numerous compared to those of metaphase, and the M/A + T ratio was 0.7−0.9. In the meristems of roots treated with NaCl, the M/A + T ratio was very low and ranged from 0.3−0.6. In this treatment, cells in prophase were prevalent; however, the number of cells in metaphase decreased.

Abnormalities

In control plants, most root tip cells underwent a typical course of mitosis without disturbance (Tab. 4). In roots of onion cultured on media with sorbitol, the total number of aberrant cells did not exceed 20%. The number of abnormal cells increased with sorbitol concentration in the medium and time of exposure. In this treatment, the most frequent abnormalities were nuclear budding (1.8−8.1%; Fig. 1o) and nuclear fragmentation (0.2−6.8). Addition of 100 mM of NaCl to culture medium resulted in the appearance of chromosomal aberrations such as sticky chromosomes (12.7%; Fig. 1f) as well as chromosomal bridges and fragments (10.1%; Figs. 1g−j). The highest percentage (46.5± 8.1) of aberrant cells was recorded in roots of plants treated with 200 mM of NaCl for 20 days. In this treatment, considerable frequency of meristem cells with lagging chromosomes (9.5%); nuclear budding (5.5%); nuclear disintegration and fragmentation (13.3%; Fig. 1p); and micronuclei (8.9%; Figs. 1k−n) was recorded. In roots exposed to iso-osmotic solutions of sorbitol, total percent of aberrant cells was lower, and nuclear budding and fragmentation were mainly observed.

Tab. 4. Cytogenetic abnormalities in *Allium cepa* root meristem cells exposed to sorbitol and NaCl *in vitro*. Means in column followed by the same letter are not significantly different ($p \leq 0.05$, Student−Newman−Keuls test). Lg – laggard chromosomes, Br/Fr – bridges and fragments, Stc – sticky chromosomes, Nb – nuclear budding, Nf – nuclear fragmentation, Mn – micronuclei.

Treatment (mM)		Exposure (days)	Chromosome aberrations (%)			Nuclear aberrations (%)			Total aberrant cells (%±SE)
			Lg	Br/Fr	Stc	Nb	Nf	Mn	
Control		10	0.0	0.0	0.0	0.0	0.0	0.0	0.0±0.0 e
		20	0.0	0.0	0.1	0.0	0.0	0.0	0.1±0.0 e
Sorbitol	100	10	0.8	0.0	0.8	8.7	0.2	0.3	10.7±3.0 ed
		20	1.2	0.7	4.1	1.8	5.3	0.2	13.3±4.4 cde
	200	10	0.3	0.0	4.5	5.7	2.0	1.3	13.8±4.5 cde
		20	2.1	2.2	1.7	4.5	5.5	1.8	17.7±4.8 bcd
	360	10	1.6	0.0	2.1	3.6	3.0	0.1	10.4±1.8 ed
		20	1.8	1.3	0.0	8.1	6.8	0.6	18.6±6.7 bcd
NaCl	100	10	8.1	5.1	3.3	1.6	0.5	0.4	19.1±4.6 bcd
		20	6.9	10.1	12.7	0.0	1.5	1.1	32.3±6.0 ab
	200	10	12.1	4.2	2.8	1.4	3.4	2.4	26.2±2.8 bc
		20	9.5	5.0	4.3	5.5	13.3	8.9	46.5±8.1 a
	300	10	4.4	1.1	2.6	0.6	4.0	2.8	15.5±0.5 bcde
		20	0.0	0.0	0.0	0.1	0.2	0.1	0.4±0.0 e

Discussion

Cell size is controlled by a hierarchy of regulatory systems that senses the overall organ size and the total mass of the organism (Kondorosi et al. 2000). The maintenance of cell size requires homeostasis between macromolecule synthesis and degradation. Maintenance is also linked to nutrient and growth factor availability and is influenced by the environment (Zonia and Munnik 2007, Osakabe et al. 2013). Changes in root meristem cell size under abiotic stress have not been deeply investigated. In this study, both sorbitol and NaCl caused a decrease in root meristem cell area, which confirms previous findings in other types of cells (Kimball et al. 1975, Zörb et al. 2015). A greater decrease in interphasic cell cross-section area was observed after salt treatment; however, comparison of the effects of iso-osmotic solutions of sorbitol and NaCl showed similar results, and the area of treated cells was reduced to approximately half the size of the control cells after 20 days of treatment. The decrease in cell size might mainly be due to the reduction in cytoplasmic volume, the loss of cell turgor being a consequence of osmotic outflow of intracellular water (Rhodes and Samaras 1994, Mahajan and Tuteja 2005). The decrease in cell cross-section area was observed from the beginning of the experiment and progressed gradually with stress agent concentration. During the entire experiment, meristem cells in roots of plants cultured on media with sorbitol and 100 and 200 mM of NaCl were mitotically active. This implies that the onion meristem cells that were reduced in size still undergo mitosis and produce new cells. Previously reported reductions in meristem size under salt and osmotic stress (Hanif and Davies 1998, Zadeh 2007, Ji et al. 2014), which suggested as being due to a decrease in mitotic activity, might be also partially due to smaller size of newly produced meristem cells.

Microscopic observations also reveal that in salt-treated onion root tip cells (200 and 300 mM of NaCl) the nucleus was shifted toward the cell periphery. During salt stress, excessive ions accumulate in the vacuole to avoid ion toxicity but also to increase cellular osmolarity to counter osmotic stress (Garbarino and Dupon 1988). Mimura et al. (2003) reported that in *Bruguiera sexangula* protoplasts subjected to NaCl an increase in vacuolar volume was observed. Although vacuole volume was not measured here, this process might explain the observed polarization of meristem cell nuclei after NaCl treatment. There are very few reports covering interphasic Vn changes in plant cells under salt and osmotic stress. The effects of salt on Vn were reported for wheat (Yumurtaci et al. 2009) using sensitive and tolerant genotypes. The results showed a reduction in Vn in roots of salt-sensitive wheat cultivars exposed to 300−500 mM of NaCl for 24 h. On the other hand, there are also studies reporting an increase in the size of nuclei under salt stress. Interestingly, such observations were made for the halophyte *Atriplex prostrata* (Katembe et al. 1998) and for a salt-tolerant cultivar of wheat (Yumurtaci et al. 2009). The present study showed that in *A. cepa*, both sorbitol and NaCl cause a decrease in the Vn of root tip cells. Comparison between the effects of iso-osmotic solutions of NaCl and sorbitol showed that the decrease was more severe after salt treatment. Such results suggest that osmotic stress resulting from sorbitol and NaCl treatment influences the Vn of root meristem cells; however, the excessive accumulation of ions in the root tips of plants cultured on media with 200 and 300 mM NaCl increases the adverse effects. The observed decrease in the Vn under NaCl-induced stress might be from nuclear deformation and subsequent nuclear degradation as a consequence of specific Na^+ and Cl^- ion toxicity, which are responsible for alterations in DNA and fragmentation of chromatin (Katsuhara and Kawasaki 1996, Yazdani and Mahdieh 2012). The observed decrease in Vn under osmotic stress is probably due to changes in hydration of the nucleus (Mitchell and Van der Ploeg 1982, Dogan et al. 2012). Sorbitol does not produce any ion toxicity; however, it has a dose-dependent ability to lower the water potential of an external solution, causing hypertonic stress, which could explain the observed decrease in both cell size and nuclear volume.

The MI in untreated (control) root tips of *A. cepa* collected from roots of small bulbs (3−4 g) placed in glass bottles filled with water ranged from 6% (Nefic et al. 2013) to 15.4% (Mesi and Kopliku 2013), while in similar experiments with large bulbs (20−60 g) the MI in root tips was close to 60% (Sehgal et al. 2006, Kumari et al. 2009). In experiments where roots were produced from small onion bulbs (1.5−2 cm in diameter) placed in boxes with sand moistened with water, the reported MI was approximately 60% (Ateeq et al. 2002, Pandey et al. 2014); however, in roots produced from small bulbs grown in Hoagland's solution, the MI was 12−21% (Glińska et al. 2007, Zou et al.

2012). In experiments where root tips were collected from onion seeds germinated in dishes with moistened paper, the MI was 17−25% (Fernandes et al. 2007, Leme et al. 2008). In the present study, mitotic divisions were scored in roots obtained from seeds germinating and growing in sterile culture media. Mitosis in the roots of control plants was normal and undisturbed and nearly 30% of observed cells undergo mitosis, which ranks among the higher limits reported for *A. cepa* MI index values, very probably a consequence of used plant material (seeds) and applied growth conditions (tissue cultures).

Applied stress affected mitotic activity in onion roots. The total absence of mitotic activity was observed after a 20-day exposure to the highest concentration (300 mM) of NaCl, while approximately 16% of the meristematic cells in roots growing on media with the highest concentration (360 mM) of sorbitol still underwent divisions during this period. Such results point to differences in the mitodepressive effect of ionic and non-ionic stress. During root growth, the number of dividing cells in meristematic tissue is related to the duration of mitosis in the cell cycle. The number of cells in a division phase is proportional to the time spent by that phase in relation to the total length of mitosis (González-Bernáldez et al. 1968). In roots treated with sorbitol, the proportion of certain mitotic phases was similar to that of the controls, while NaCl treatment significantly altered the phase ratio. NaCl treatment decreased the number of cells in the metaphase and increased the number of anaphases and telophases, while in the controls almost equal proportions of metaphase and ana/telophase cells were observed. The accumulation of anaphase and telophase, which is evident from the low (0.4) M/A + T ratio, may be a consequence of the weakened chromosome separation, probably by stickiness of chromosomes (Tajbakhsh et al. 2006). In addition, after stress treatment the cells entered mitosis, but they were arrested in prophase, and the cell cycle often did not progress further, as was observed in control cells. These results suggest an increase in duration of the cell division cycle under applied stress conditions and a stronger mitodepressive effect of salt stress in comparison with sorbitol-mediated osmotic stress. In the roots of salt-tolerant genotypes, no significant changes in MI between treated and control samples were observed (Radić et al. 2005). Thus, the level of mitotic activity reduction in root meristem cells under stress might be a specific marker for discrimination between salt-sensitive and salt-tolerant genotypes. Detailed cytological analysis of sorbitol- and NaCl-treated onion root tip cells allowed for characterization of numerous abnormalities in interphase and during cell division. The frequency of aberrant cells differed with the stress agent and its concentration. In salt-treated roots, the highest percent of aberrations (approximately 46%) was observed in roots of plants grown on medium treated with 200 mM of NaCl. In those cells, considerable frequencies of chromosomal aberrations, nuclear fragmentation and micronucleated cells were observed. In root meristems of plants exposed to iso-osmotic solutions of sorbitol, the percent of abnormalities was lower and accounted mainly for nuclear aberrations. Observed chromosome fragmentation and chromosome breaks indicate clastogenic action, while chromosome stickiness may be a result of inter-chromosomal linkages coupled with excessive formation of nucleoproteins (Leme and Marin-Morales 2009). Lagging chromosomes resulted most likely from the failure in organization of spindle apparatus (Tabur and Demir 2009, Utani et al. 2010). The formation of micronuclei, observed here as prevalent in NaCl-treated cells, may be a consequence of chromosome fragments or lagging chromosomes failing to incorporate into the daughter nuclei (Yi and Meng 2003).

In conclusion, the effects of different osmotica on plant tissues depend on the physical and chemical nature of stress-inducing agents. Sorbitol is an osmoticum that does not induce ion toxicity, although influences the water balance within the cell. Results obtained in this study suggest that onion root meristem cell size is mostly affected by changes in extracellular osmolarity, while nuclear size is affected both by ionic and osmotic components of applied stresses. Both stress agents caused a decrease in mitotic activity and increased the number of mitotic abnormalities in root tip meristems of onion, albeit with various dose- and exposure-dependent frequencies, which points to differences in their cytotoxicity. Comparison of effects of iso-osmotic NaCl (200 mM) and sorbitol (360 mM) concentrations showed that the ionic rather than the osmotic component of salt stress had more detrimental effects on onion root meristems.

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